

## LEISHMANIA BRAZILIENSIS IN THE PANAMANIAN TWO-TOED SLOTH, *CHOLOEPUS HOFFMANNI*\*

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**Abstract.** A total of 498 two-toed sloths, *Choloepus hoffmanni*, collected in central Panama was examined for *Leishmania braziliensis* over a 10-year period. Isolations of the parasite from 96 (19.3%) of the animals were confirmed by culture and inoculation of golden hamsters. Improved culture techniques developed toward the end of the study assisted in determining a greater prevalence of the disease. Infections were completely cryptic in all animals, and the parasite was isolated from skin, blood, liver, spleen, bone marrow and lung tissues. Sloths maintained under seminatural conditions remained infected up to 23 months, the longest period of survival. This edentate, considered the principal reservoir host of *L. braziliensis* in Panama, showed infection rates from 0-59.4% in various communities, which appeared to correlate with the parasite prevalence in the indigenous human populations.

Investigations on natural leishmanial infections among Panamanian forest mammals were conducted by the authors from March 1965 to December 1974. Three different dermatotropic species of *Leishmania*, *L. braziliensis*, *L. mexicana* and *L. hertigi* were found in 13 species of feral mammals of the orders Edentata, Marsupialia, Primates, Rodentia and Carnivora. Principal findings up to 1972 have been reported in a previous publication.<sup>1</sup>

The two-toed sloth, *Choloepus hoffmanni*, was first found infected with *L. braziliensis* in February 1968. Thereafter, this edentate consistently showed the highest infection rate among all the forest mammals in which natural leishmanial infections were demonstrated. Ecological and epidemiological investigations<sup>2</sup> showed a close relationship between the geographic distribution of human cutaneous leishmaniasis and the presence of leishmanial infection in the two-toed sloth, and we consider this edentate as the principal reservoir host of the human disease in the central part of Panama.

The present paper deals with the nature and course of the infection in two-toed sloths.

### MATERIALS AND METHODS

A total of 522 *C. hoffmanni* was processed at Gorgas Memorial Laboratory (G.M.L.) during our studies; 498 were collected in different localities of the central part of the Isthmus. The remainder (24) comprised 10 laboratory-born specimens and 14 fetuses. Animals were collected by our own field personnel as well as by local collectors. The age of the animals was determined arbitrarily on the basis of weight, as follows: young, up to 1,999 g; juvenile, 2,000-3,999 g; adult, 4,000 g and over.

### Maintenance of sloths in captivity

We attempted to keep the sloths alive as long as possible in order to examine them repeatedly. During the first phase of the studies they were maintained in a 4 × 6 meter outdoor cage with two sides and half of the roof fenced with wire mesh. Carrots, oranges, yucca, and lettuce were provided, and occasionally fresh leaves of *Cecropia* spp. and mango fruit were offered. Beginning in September 1971, a large enclosure in Panama City was prepared for the sloths. This was an area of 59 × 61 meters, surrounded by a high cinder block wall. In addition to three large mango trees at this site, a number of *Cecropia*, plum, and papaya trees were planted. These trees, and the herbaceous vegetation, provided a seminatural environment for the sloths.

The enclosure was in the central part of the city, far from known breeding sites of sand flies. On four occasions CDC miniature light traps were set up during the night, both at ground level and

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TABLE 1

Flagellates isolated in culture from 260 of 498 two-toed sloths, *Choloepus hoffmanni*, in Panama

Species	No. (%) infected
<i>L. braziliensis</i>	45 (9.0)
<i>L. braziliensis</i> and <i>E. schaudinni</i>	36 (7.2)
<i>L. braziliensis</i> , <i>E. schaudinni</i> , and <i>T. rangeli</i>	3 (0.6)
<i>L. braziliensis</i> , <i>E. schaudinni</i> , and <i>T. cruzi</i>	1 (0.2)
<i>L. braziliensis</i> and <i>T. rangeli</i>	11 (2.2)
<i>E. schaudinni</i>	83 (16.7)
<i>E. schaudinni</i> and <i>T. rangeli</i>	24 (4.8)
<i>T. rangeli</i>	57 (11.4)
Total sloths found infected	260 (52.2)

at different heights in the trees, to determine the possible presence of sand flies.

#### Processing of the sloths

Following the tissue-biopsy-culture technique previously described,<sup>3</sup> cultures from heartblood and skin were made from the sloths on their arrival at G.M.L. and repeated periodically during the time the animals survived in captivity. The same procedure was performed on laboratory-born animals. Fresh blood was also examined microscopically for the presence of other hemoflagellates. Thin and thick blood smears were prepared from animals infected by trypanosomes or *Endotrypanum schaudinni*. Samples of skin, heartblood, liver, spleen, and bone marrow were cultured at autopsy. Impression smears were made from the liver and spleen, fixed in methanol and stained with Giemsa.

#### Isolation and characterization of

##### *L. braziliensis*

Flagellates isolated in culture were routinely inoculated intradermally ( $5-10 \times 10^6$  organisms) in the nose of 5-8 golden hamsters, *Mesocricetus auratus*, which were maintained under close observation as long as possible. *L. braziliensis* usually produced a characteristic swelling after 3-4 weeks at the site of inoculation, which contained the tissue form (amastigote) of the parasite. Hamsters which did not show any skin alteration for 3 months or more were killed and skin cultures were made from the nose; such animals rarely were found to be infected with *L. braziliensis*.

TABLE 2

Sources of *Leishmania braziliensis* isolation in culture from the two-toed sloth, *Choloepus hoffmanni*, in Panama

Tissue	No. examined	No. positive	% positive
Blood	439	9	2.1
Skin	497	56	11.3
Nose	483	38	7.9
Chin	457	18	3.9
Ears	467	11	2.4
Ventral skin	126	2	1.6
Feet	166	2	1.2
Back skin	93	0	0
Others	9	0	0
Viscera	451	54	12.0
Spleen	451	46	10.2
Liver	450	26	5.8
Bone marrow	382	14	3.7
Lungs	267	4	1.5

Characterization of *L. braziliensis* was made on the basis of the infectivity to the golden hamster and the course of the infection in this rodent.<sup>1</sup> Criteria used in the identification of *E. schaudinni* in culture were its lack of infectivity for the golden hamster and the frequent presence of promastigotes with significant elongation of the posterior end, which have been called "long-tailed" forms.<sup>4</sup> Criteria followed to characterize the other hemoflagellates isolated in culture have been described previously.<sup>5</sup>

## RESULTS

#### Trypanosomatidae isolations

Trypanosomatids frequently occurred as multiple infections, which complicated the isolation and identification of *L. braziliensis*; Table 1 shows the frequency of isolation.

The following Trypanosomatidae were isolated in culture from 498 wild-caught Panamanian two-toed sloths: 96 (19.3%) *L. braziliensis*, 147 (29.5%) *E. schaudinni*, 95 (19.0%) *Trypanosoma rangeli*, and 1 (0.2%) *T. cruzi*.

#### *L. braziliensis* isolations

*L. braziliensis* was cultured from blood, skin and viscera of wild-caught two-toed sloths. The number of cultures made from each site and the results are presented in Table 2 for the 498 ani-

TABLE 3  
Relation of leishmanial infection to age of sloths

Age of sloths	No. examined	No. infected	% infected
Fetus	10	0	0.0
Laboratory-born	14	0	0.0
Young	56	16	28.6
Juvenile	103	23	22.3
Adult	339	57	16.8
Totals	522	96	18.4

mals collected. Isolations of *L. braziliensis* in cultures from 95 infected sloths showed the following pattern of parasite distribution: 32 (33.7%) from skin only, 40 (42.1%) from viscera only (includes blood and bone marrow) and 23 (24.2%) from skin and viscera.

#### Leishmanial infections in relation to age

Prevalence of *L. braziliensis* among sloths grouped by age showed that infection rates were inversely proportional to the age of the animals (Table 3). Of the nine animals with positive blood cultures (Table 2) infection rates of 3.8%, 2.9% and 1.2% were recorded in young, juveniles and adults, respectively. In addition to the 498 wild-caught sloths examined, 10 fetuses and 14 laboratory-born animals were processed. *L. braziliensis* was isolated from four of the 10 fetuses from which the fetuses were obtained and three of the 14 mothers of the laboratory-born animals; all of the fetuses and newborn animals were negative for leishmaniasis.

#### Duration of infections

Of the 498 sloths collected during our investigations, 63 of 371 (17.0%) animals, examined within a few days after arrival at G.M.L. or up to 5 months of captivity in the small enclosure, were positive for leishmaniasis (Table 4). The remaining 127 sloths were released in the large enclosure and 12 of 46 (26.1%) of these animals, which died and/or were examined after less than 1 month in captivity, were positive for leishmaniasis. *Leishmania* was isolated from 21 of the other 81 (25.9%) animals which survived from 1-23 months in captivity in the large enclosure.

Four sloths had negative skin cultures on arrival, although parasites were detected during their captivity. The infections in 10 sloths were dem-

onstrated only at autopsy from cultures of the viscera. In five (83.3%) of six animals which survived in captivity from 21 to 23 months the infections persisted until they died, or were killed. Nine sloths in which *L. braziliensis* was isolated from skin samples taken within a few days of capture became *Leishmania*-free after 1-16 months of captivity (average 5 months).

Phlebotomine sand flies were absent in all light trap collections in the large sloth maintenance area, thus precluding transmission subsequent to capture. Phlebotomines have never been reported from this central section of Panama City.

#### Search for amastigotes

Smears from 400 blood, 328 viscera and five skin lesion samples were examined for vertebrate parasite forms (amastigotes) of *L. braziliensis*. Amastigotes were found only in 14 smears from visceral organs (particularly the spleen). A few amastigotes were seen in the cytoplasm of leucocytes of two young sloths. In the other 12 cases amastigotes were very rare, free, and poorly stained.

#### Improvement of culturing techniques

From 1968-1971 single tissue samples from skin and/or visceral sites were cultured from each sloth. Because of the complete absence of any gross indication of the infection in this edentate, and the isolation of the parasite from random areas of the skin, the number of samples for culture from each site was increased approximately three-fold. The increased tissue sampling resulted in the detection of infections in a greater proportion of animals, as shown in Table 5.

The occurrence of infections among sloths in various areas of central Panama showed a full range of endemicity as demonstrated by the following prevalence rates: Chilibre and Chagres none of 22 (0%), Chorrera four of 61 (6.6%), El Aguacate 38 of 153 (24.8%) and Las Tablitas 19 of 32 (59.4%). No leishmanial scars or lesions were observed among the inhabitants of Chilibre. In 1970, 73 of 174 (42.0%) of the people examined in El Aguacate had leishmanial scars or active lesions.<sup>2</sup> Six of 23 (26.0%) sloths examined during the same year from this village were infected with *L. braziliensis*. In the small village of Las Tablitas the majority of members of several families visited had leishmanial scars or active lesions. Endem-

TABLE 4

Prevalence of *Leishmania braziliensis* among 498 two-toed sloths collected in Panama from 1965 to 1974

Conditions and periods of captivity	No. sloths examined	No. sloths positive	% sloths positive
Small enclosure (4 × 6 m): sloths examined immediately or up to 5 months in captivity	371	63	17.0
Large enclosure (59 × 61 m):			
a) Sloths died and/or were examined after 1 month in captivity	46	12	26.1
b) Sloths died and/or were examined after 1–23 months in captivity	81	21	25.9
Total	498	96	19.3

icity of the disease, as reflected by the prevalence of *L. braziliensis* in sloths, ranged from nonzootic to epizootic and appeared to correlate well with the prevalence of the disease among the indigenous human populations of these areas.

## DISCUSSION

The cryptic nature of *L. braziliensis* infections in the two-toed sloth, and the occurrence of concomitant infections with other hemoflagellates, complicated determinations of leishmanial infection rates in this animal. Although utilization of the skin-biopsy-culture technique<sup>3</sup> facilitated the sampling of skin, viscera and blood, the flagellates cultured always required careful characterization. Intradermal inoculation of promastigotes in the nose of the golden hamsters has proved to be a reliable technique, since the infectivity and characteristic local reaction produced in the rodent by *L. braziliensis* differentiates this parasite from *E. schaudinni*, *T. rangeli* and *T. cruzi*.

In a recent publication Zeledon et al.<sup>6</sup> described a new *Leishmania* species, *L. herreri*, isolated from *C. hoffmanni*, *Bradypus griseus* and three phlebotomine sand fly species in Costa Rica. Al-

though the new species was not infective to hamsters it produced amastigotes in primary hamster embryo tissue cultures incubated at 33°C, a criterion used by the Costa Rican workers to differentiate the new taxon from *Endotrypanum* which does not produce amastigotes in tissue culture.

Since Costa Rica and Panama share a common border, and *L. herreri* was isolated from *C. hoffmanni*, we have initiated tissue culture and isozyme studies of all promastigotes isolated from sloths which are not infective to hamsters to determine whether *L. herreri* occurs in this country.

All of the isolates from *C. hoffmanni* characterized as *L. braziliensis* in the present study were infective to hamsters.

We are not aware of any reports of transplacental transmission of *L. braziliensis* among reservoirs; the occurrence of such a phenomenon among Panamanian sloths appears unlikely since seven progeny (four fetuses and three newborns) of infected females were *Leishmania*-free, based on the tissue-biopsy-culture technique. Our data indicate, however, that the age of two-toed sloths is an important factor in leishmanial infections since the infection rate was higher in young animals than in juveniles and adults (Table 3). It

TABLE 5

Improved detection of natural infections of *Leishmania braziliensis* in two-toed sloths resulting from increased tissue sampling in culture techniques

Study localities	February 1968–May 1971			June 1971–December 1974		
	Sloths processed	Found infected	Infection rate	Sloths processed	Found infected	Infection rate
Achiote	21	5	23.8	8	4	50.0
Aguacate	98	20	20.4	55	18	32.7
Chorrera	33	2	6.1	28	2	7.1
Las Tablitas	16	8	50.0	16	11	68.8
Madden	20	2	10.0	6	1	16.7
Totals	188	37	19.7	113	36	31.8

appears probable, therefore, that in endemic areas sloths acquire the infection during the first few months of life and remain infected for long periods of time. Although sloths may remain infected for 2 years or more, the disappearance of parasites in some animals indicates that infections may be self-limiting. The possibility that such animals are then immune to reinfection may explain, in part, the substantially lower infection rates among older animals despite their accessibility to phlebotomine vectors.

We do not know whether concomitant infections with other hemoflagellates affect the course of leishmaniasis in sloths; however, we have isolated *L. braziliensis* 17-23 months after capture from five animals which had concomitant infections with *E. schaudinni* or *T. rangeli*.

The improved culturing techniques utilized to isolate *L. braziliensis* from sloths and other sylvatic animals have shown that the prevalence of leishmaniasis in the edentate is substantially greater than our early studies indicated. This discovery lends additional credence to our theory that *C. hoffmanni* is indeed the principal reservoir host of *L. braziliensis* in Panama, and that the seven additional indigenous sylvatic animals from which we have isolated the protozoan represent ancillary hosts.<sup>1</sup>

The isolation of *L. braziliensis* from skin, blood, spleen, liver, bone marrow, and lung tissues of sloths without evidence of pathology to the animal indicates a long association between reservoir and agent which appears to have evolved to a commensal relationship.

During the course of our studies it became apparent to us that there was a correlation between

infection rates among sloths and in villagers. Recognition of the existence of such a correlation may prove valuable to future environmental impact studies for activities such as road building or dam construction in remote sylvatic areas. Determining the prevalence of leishmaniasis among sloths in such areas may provide a potential "infection rate index" for workman at risk.

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